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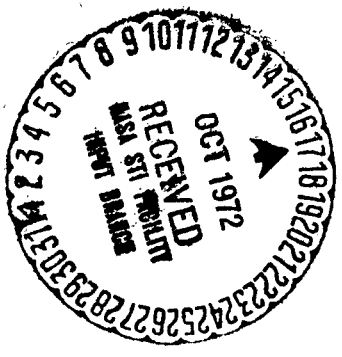

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WATER-SOLUBLE FILTERS FOR BACTERIAL COUNTS OF THE AIR

ABSTRACT

by

K-H. Maier and K. Voggel.

Our report describes a new filter material, based on such water-soluble sustances as gelatine, for determining the bacterial content of the air. Materials and methodology were developed outside and largely independent of laboratory facilities in view of efficient application in practice of the method. The bacteria including yeasts and fungi carried by the air belong with their small dimensions of a few 1/1000 mm. to the class of suspended matter not subjec to sedimetation. Consequently, special methods of collection are needed for their detection. However, under conditions in nature, it can be assumed that these bacteria generally do not exist as separate individuals but in agglomeration and association with finely-divided dust of all kinds.

Unusual difficulties are presented by the analysis of suspended inorganic matter due to air currents, the prevailing turbulence and the minute division of the particles and these are further increased in examination for bacterial content. These increased difficulties result from the necessity of taking into consideration all prerequisites of bacteriological methods of investigation, especially sterile methods of

obtaining the specimens in the field and the later culture of the captured bacteria on appropriate bacteriological nutrient media. A comparative report on the results of various methods of determining the bacterial content of air was published in ref. 1 and represents the state of investigative techniques at the time.

Table 1 represents the methods of examination generally used previously:

Table 1

Methods of Examination	Principles
Placing on nutrient-media	Sedimentation
Forcing an air stream over nutrient media	"Conimetry"
Passage of an air stream through a layer of fluid	Impinger
Passing an air stream trough a filter by suction	Filtration

Any method for determination of bacteria consists in principle of two separate steps, the collection and concentration of the suspended bacteria from an air space over a narrowly limited surface and/or in a relatively small volume of fluid, and of the subsequent identification primarily through culture by incubation.

For the methods of filtration exclusively discussed here, it appears necessary to state some basic requirements for practical application:

1) the filter material used for bacteria collection must be suitable for quantitative precipitation of the bacteria from the air. On the other hand, it must possess high permeability to air in order to make possible briefinterval bacteria collection or the throughput of large volumes of air:

2) the bacteria collected on the filter must be capable of being incubated in order to make possible quantitative evaluation:

3) the method of collecting the bacteria should not be restricted to the laboratory and be possible anywhere.

Specifically in the field of filters, two methods have been proposed in recent years whose particular character and the results obtained with them should be discussed here for comparison with the water-soluble filters developed by us. These other methods are the use of commercially available membrane filters on the basis of water-soluble sodium "alginate".

In regard to membrane filters, their excellent suitability for microbiological investigations of fluids such as water, milk and others has long since been known. The character of the membrane structure as microporous foam system guarantees high capability for retention even for the most minute particles due to very narrow and defined pore widths. Simultaneously, membrane filters possess excellent permeability for flowing media due to an extremely high number of pores per unit area. These excellent properties have proved themselves also in the examination of aerosols.

The first examination of bacteria of the air with this material were made in 1948 by ref. 2. The original hopes for the suitability of this material for investigation of the air have unfortunately not been confirmed in practice. Detailed investigations in ref. 3 showed that the incubation of bacteria collected on membrane filters is highly problematical. The viability of the bacteria was shown to vary within wide limits and to be generally completely inadequate.

The problematics in the use of fiber filters of sodium alginate are

of an entirely different kind. As will be shown, the capability of retention for bacteria of the sodium-alginate fiber filters--- permeability for air comparable to membrane filters -- is disappointingly low.

This is probably explained by the relatively coarse fiber structure of these filters. Our particular interest in this filter type was the possibility of dissolving the filter together with its load of bacteria in aqueous media.

The negative experiences with the filters here described furnished inducement for the development of a new filter type. The new filter type was to have the obvious advantages of membrane structure, i.e. high efficiency and performance, but should not deteriorate the viability of the captured bacteria. We sought the solution of this problem in developing membranes with high permeability to air on the basis of water-soluble membranes in no way different in efficiency and performance from membrane filters (cellulose esters), can be obtained also on a basis of hydrophile substances. Basically suitable for this are hydrophile polymers, independent of natural or synthetic origin. Examples are gelatine, methyl cellulose and polyvinyl alcohol. For reasons of convenience in manufacture, we decided on the type of the gelatine filter.

The preparation of the gelatine membrane (ref.4) starts with an aqueous gelatine solution to which are added structure-forming additives such as ethyl alcohol. This mixture is spread in a film uniformly on a plane support and subjected to partial gelation.

Water is removed from the gelatine gel in a bath of water-miscible organic fluids. The membrane formed is dried carefully.

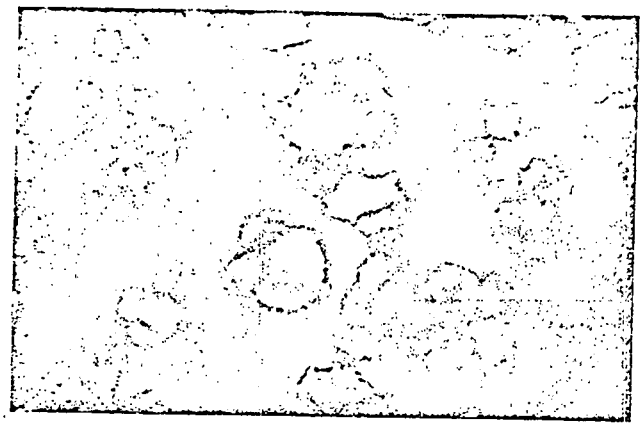


Fig. 1. Structure of gelatine membrane (200 x), transilluminated microphoto.

We give below the most important characteristics of gelatine membranes obtained by the above method. Gelatine membranes are thin white discs with a thickness of 250 micron; the weight is about 8 mg/cm^2. They possess a pore volume of 80 to 85 % by reason of their loose structure. In principle they can be obtained in all degrees of fineness appropriate for the analysis of air. However, we shall restrict ourselves to the example of a filter type proved to be particularly suitable for the collection of bacteria.

This type has a permeability for air of 2.5 lit per cm^2. per min at 500 mm H_2O. This corresponds to the permeability of membrane filters widely used in Germany and USA for aerosol analyses. This permeability is due to the extremely high number of pores per cm^2 of filter surface of about 10^7. The mean pore diameter is 1 to 3 micron. Since experience has shown that the capability for retention of suspended matter of the air is much higher than corresponds to the mean pore diameter, complete retention of all particles of one micron or less can be expected. In practice, this type of filter has proved to be impermeable for bacteria. Gelatine membranes can also be sterilized with ethylene oxide for bactriological application

but are furnished already sterilized by the manufacturer.

The most important properties meriting special stress appear to be the ideal capability of dissolution in aqueous media and maintainance of the capability for incubation of the bacteria collected on the gelatine membranes. Table 2 contains as a demonstration of the preserved viability of the bacteria an example for the bacterial counts obtained by us after filtration of room air through gelatine membranes. For comparison, the results of bacterial counts from membrane filters (cellulose ester) and with the impinger are listed:

Table 2

Filter Type	Bacterial Count
Gelatine membranes	480
Impinger	500
Membrane filter	8

These bacterial counts were obtained by us under the following conditions.

Gelatin membranes with the permeability specified above and a mean pore diameter of 2 micron were sterilized with ethylene oxide and clamped in an also sterilized device for filtration. A filter diameter of 50 mm furnished an effective filter surface of 15 cm^2. 100 lit. of air were passed through this membrane at a negative pressure of 250 mm H_2O for an interval of 5 min.

For comparison, commercially available membrane filters were utilized for filtration of air under the same conditions and an air strean of the

same volume conducted through a customary impinger as control. In all cases, cultures of the collected micro organisms were made in a known manner.

This example is typical for the differences in the bacterial counts from membranes of hydrophile and hydrophobe character. Counts on membrane filters are definitely less, frequently by one to two orders of magnitude, than on gelatine membranes. By contrast, values from gelatine filters and impingers agree well.

The superior preservation of the viability of the bacteria is in our opinion due to the fact that the bacteria deposited on the gelatine membrane or within the filter structures are not exposed to dehydration to the same extent as on cellulose-ester memebranes. It must be assumed that this is linked to the residual water content of the gelatine memebrane which this material retains during preparation and/or a certain subsequent absorption of moisture. It is possible that this water content affords a certain protection against dehydration.

All of the presently known methods in bacteriology can be utilized with certain variations for growing the bacteria on gelatine filters.

The simplest method consists in transfering the membrane with the collected material to a Petri dish and to add sufficient nutrient solution for steeping of the membrane (porous cardboard is recommended as support for the membrane). Steeping transforms the membrane into a semi-solid gelatinous culture medium which can be used in customary manner in the incubator for growing of bacterial colonies. The colonies are easily distinguished and can be counted after incubation.

The solubility of the gelatine membrane in aqueous media affords

further possibilities. For this purpose, a bacteria-charged membrane (50 mm in diameter) is transferred to a sterile test tube with a diameter of 30 mm and the addition of 40 ml of sterile water, physiological sodium-chloride solution or eventually a nutrient solution. The closed tube is then left for 30 min in a thermostat at 30°C. During this time takes place a complete and uniform disolution of the membrane producing a bacterial suspension.

The liquid bacterial concentrate can then be cultured in any desired manner like any other bacterial preparation. The complete dissolution of the gelatine filter possesses some advantages. It is very probable that the rapid transfer of the collected bacteria to a liquid medium is particularly advantageous for the preservation of their viability.

Such a suspension can also be divided into several parts, each of which permits further work by different methods of culture with different specific or non-specific nutrient media or different incubation conditions. We consider as a particularly appropriate method of culture for the bacteria in such a suspension a method which is in general use under the designation "membranefilter method" for bacteriological water tests. The bacterial suspension is here filtered through a sterile membrane filter of type Co5(cellulose ester) where the bacteria are deposited on the filter surface. After filtration, the Co5-filter is placed on a nutrient medium, e.g. the usual agar preparation or nutrient-saturated cardboard, and incubated in the incubator. We have used this method generally in our experiments. In order to obtain a critical comparison of results with gelatine and cellulose-ester membranes as well as sodium-alginate filters respectively, we carried

out tests under defined conditions in an aerosol chamber.

In order to obtain an aerosol suitable for bacteria, relative moisture was adjusted to 90 % by atomizing distilled water in an air chamber at 20°C. Subsequently, 4 ml of a bacterial suspension of Sarcina lutea and Staphylococcus albus, both grown from air, from an elutriation with physiological sodium-chloride solution in a slanted test tube were atomized in the chamber. The bacteria were respectively 5 days and 24 hours old. The bacterial suspension consisted of a mixture of two bacteria at a ratio of 1:1. Immediately adter spraying, the bacteria were uniformly distributed by shaking. After sedimentation for 30 min, the various filter types were tested with the bacterial preparation. The impinger method was again used as control.

Comparison of Gelatine and Alginate Filters

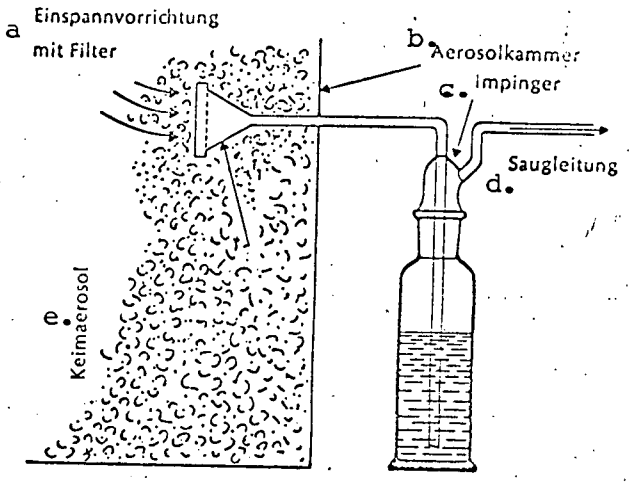
We were primarily concerned in comparing the capability of retention for bacteria of the air in the filtration process of the two filter types. Both types have the solubility in aqueous media in common but differ essentially in the structure, degree of fineness and thickness of the filter.

Fig. 2. Structure of fiber filter (300 x).

The alginate filter (ref.5)was prepared by us strictly in accordance with the data in literature. Fibers of calcium alginate with a length of 5 to 10 mm were treated with hydrochloric acid, washed and neutralized with sodium hydroxide. The resulting product was again washed and dried with absolute alcohol. The sodium-alginate filters thus obtained had a thickness of 4 mm with a weight of 100 mg/cm^2. The microscopic picture of this fiber filter indicated an average fiber thickness of 10 micron. The interstices between the individual fibers were between 20 and 100 micron.

During inoculation of the bacteria, both the alginate filters and the gelatine membranes were held in clamping devices(effective filter surface 15 cm^2) and the air from the aerosol chamber containing the bacteria was passed through the filters by suction. Adter passing through the filter, the filtered air stream was conducted through an impinger in order to control filter efficiency. Within 10 min, 100 lit of air passed through each filter type and subsequently through the impingers. Control of the impinger fluid for escaped bacteria was made after 30 min with the aid of the membrane-filter method. For this purpose, the liquid was filtered through a sterile Co5 membrane filter and incubated on an agar-culture medium at 30° C. for 48 hours.

Examination of the impinger fluids disclosed that no bacteria of the two types could be demonstrated adter passage of the air through the gelatine membranes whereas several thousand bacteria of both types were found in each case with the use of sodium-alginate filters. These data are derived from two separate tests made in each case. It was further determined that the bacteria retained in the sodium-alginate filters amounted to only the thousandth part of the microorganisms collected on gelatine membranes.



a= Clamping device with filter.

b= Aerosol chamber.

c= Impinger.

d= Suction line.

e= Baceterial aerosol.

Fig. 3. Arrangement for demonstration of impermeability to bacteria of the filter.

Comparison of Hydrophile Gelatine and Hydrophobe Membrane Filters

Also in the air chamber, a number of comparative tests of gelatine and membrane filters was made. These tests served for confirmation of the data reported above for the growing of bacteria obtained by filtration of room air under better defined conditions in the laboratory. In the following example, the bacterial aerosol was prepared in the chamber in the same manner as described above. However, the air specimens were used here after a prolonged interval of sedimentation so that the bacterial concentration was appreciably lower. Both filter types, of the same permeability and same pore width, were clamped in the devices described. At a negative pressure of 200 mm H20, 100 lit air were drawn form the chamber and the same volume of air conducted parallel through an impinger as control.

After charging with bacteria, the gelatin filter was dissolved in sterile physiological sodium-chloride solution, filtered through a Co5

membrane filter (which was also done with the impinger fluid), placed on an agar-culture medium and incubated at 30°C. for 48 hours. The hydrophobe membrane filter utilized for air filtration was place directly on the culture medium and incubated in the same manner. This produced the following bacterial counts:

Table 3

	Membrane filters	Impinger	Gelatine membrane
First experiment	8	75	90
Second experiment	3	85	80
Mean value	6	80	85

The bacterial counts obtained form the air-chamber tests correspond insofar to the findings with room air (cf.Table 2) as they also show the concordance of the counts between the gelatine filter and the impinger. By contrast, the membrane-filter values are decisively lower.

The relation of bacterial counts form the different filter types found by us was further confirmed through different personal communications. The deviation of the bacterial counts is due to the more or less pronounced deterioration of the viability of bacteria collected on membrane filters. The degree of deterioration is in our opinion dependent, in addition to purelyclimatic factors. also on the individual sensitivity to dehydration of the types of bacteria.

We already emphasized at the beginning of our report that we attempted a practically useful method with our development and briefly outlined the requirements ofr such a method which we believe necessary. The possibilities of application appear to us manifold. Examples are the

control of room air in the pharmaceutic, food and beverage industries, in hospitals and sanatoria, and in similar installations. However, the method should also in principle include the possibility of bacterial tests in the field, i.e. any completely or partially enclosed spaces.

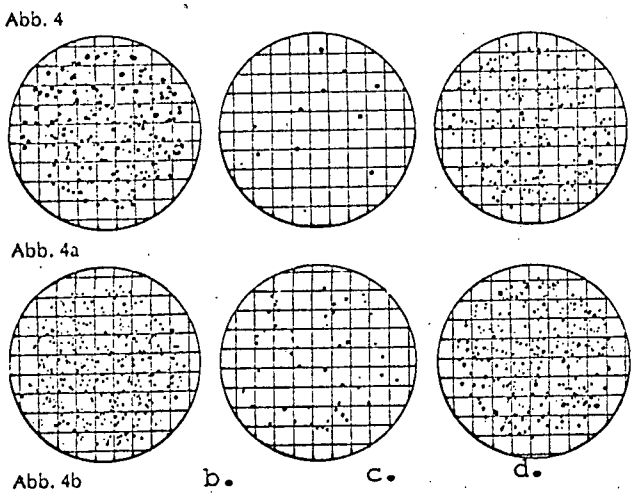


Abb. 4

Abb. 4a

Abb. 4b

a. Keimanreicherung auf:	b. Gelatine-Membran	c. Membran-Filter	d. im Impinger
e. übertragen und bebrütet auf:	f. Membran-Filter Co5	g. direkt bebrütet	h. übertragen u. bebrütet auf Membranfilter Co5
i. 4a Gefundene Keimzahl	197	10	225
i. 4b Gefundene Keimzahl	299	51	301

a = Bacteria collectedon.

b = Gelatine membrane.

c = Membrane filter.

d = Impinger.

e = Incubated on.

f = Co5-filter.

g = Direct.

h = Co5-filter.

i = Bacterial count found.

Fig. 4. Comparison of bacterial count after collection on gelatine membrane, membrane filter and impinger.

As different as may be the viewpoints under which the tests are made, sampling of the bacterial content of the air at any desired location requires in every case working under sterile conditions. Gelatine filters are delivered sterile and in sterile packing and can therefore be easily carried along to the location of sampling. Utilization of bacteria-tight

filters presupposes, because of their fineness and the consequent resistance, that these filters are clamped in suitable devices so that the filter edge is hermetically sealed. Generally of metal, such devices have threaded convers and thus make possible the insertion or removal of the filters. However, the clamping devices must be re-sterilized before each filter change. Where individual tests are concerned, several such devices previously sterilized in the laboratory can be carried along to the location of sampling. It is recommended to insert the sterile filter in the laboratory at the same time. In routine investigations where it is intended to make a large number of tests, considerable difficulties result because the device must be re-sterilized prior to every filter change. In this case, the expediture necessary for sterilization and maintenance of sterility would be difficult to support.

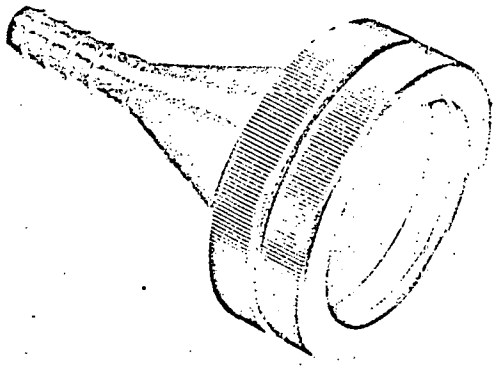


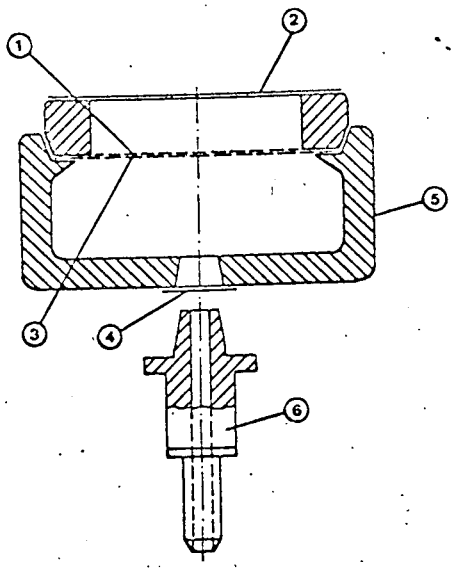
Fig. 5. Clamping device for air filters (photo by Membrane-Filter Company, Göttingen).

In order to obviate these difficulties, we developed a simple and inexpensive disposable plastic filter capsule (ref. 6). It consist of two

threaded parts tightly sealing the filter edges which contain the filter membrane and its support. Air intake and discharge apertures are closed by foil impermeable to bacteria but permeable to gas. Consequently the capsules can be gassterilized and can be taken along in any desired number.

For use, the protective foils are removed and the capsule is placed with the discharge opening on a tube leading to the suction pump. After taking the air sample, the bacteria-charged filter can be processed in the manner described above.

The capsules with the gelatine filters utilized can be taken back to the laboratory in sterile containers or can be dissolved in the field in a plastic container with sterile physiological sodium-chloride solution. The latter method also affords the possibility of processing the bacterial suspension with the already described memebrane-filter method after dissolution of the gelatine filter. In conjunction with a portable laboratory unit and of nutrient-saturated cardboard (dry culture media), further processing can also be carried out for the most part at the location of sampling. The bacterial gelatine-filtered solution is filtered through a Co5 membrane filter and the latter placed on the moistened nutrient cardboard. This is best made in Petri dishes of metal. Investigations in ref. 7 and 8 have confirmed the suitability of the portable laboratory unit for testing of water, especially in the open country. Incubation and growing of the colonies will, however, still be made generally in the laboratory. However, rapid contact of the membrane filter with the moist nutrient medium is an appreciable advantage for bacterial growth in any case.



1 =Gelatine filter.

2= Protective foil.

3= Support.

4= Protective foil.

5= Filter housing.

6= Connection to suction line.

Fig. 6. Diagram of air-filter capsule.

The gelatine-filter material and method described as developed by us has produced new possibilities of dealing with the particular difficulties of the bacterial testing of air ourside of the laboratory. We believe the advantages of our method to be not only in the fact that the gelatine filter guarantees rapid collection and complete impermeability to bacteria as well as quantitative evaluation of the bacterial counts since no deterioration of the capability for incubation of the bacteria takes place but also in the practical usefulness by relieving the investigatior through the previously effected sterilization of filter material, devices, nutrient media and other aids.

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